



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D.C. 20460

OFFICE OF  
PESTICIDES AND TOXIC  
SUBSTANCES

**MEMORANDUM**

**SUBJECT:** Methyl Bromide Rainbow Trout Acute Toxicity Test

**FROM:** *for* Anthony F. Maciorowski, Chief  
Ecological Effects Branch  
Environmental Fate And Effects Division

*Douglas J. Wilson*  
*10/12/84*

**TO:** Larry Schnaubelt, Product Manager  
Special Review And Reregistration Division

Dear Mr. Schnaubelt:

Attached is the EEB data evaluation record for an acute toxicity test for rainbow trout exposed to methyl bromide. Because of high volatility, the study design used deviated from standard protocols. The study was categorized as supplemental, but will not need to be repeated. The calculated 96 hour LC50 for methyl bromide was 3.9 mg a.i./L (C.I. 2.9 to 4.6 mg a.i./L) and this material is **moderately toxic** to rainbow trout. If you have further questions, please contact Tom A. Bailey (305-6666).



2010703



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CONCURRENCES							
SYMBOL	7507C	7507C	7507C				
SURNAME	T. Bailey	Craen	10/11/94				
DATE	9-27-94	10/11/94	10/12/94				

EPA Form 1320-1A (1/90) Printed on Recycled Paper OFFICIAL FILE COPY

9/27/94

DP Barcode : D198212  
PC Code No : 053201  
EEB Out : 9-27-94

To: Larry Schnaubelt  
Product Manager  
Special Review and Reregistration Division (H7508W)

From: Anthony F. Maciorowski, Chief  
Ecological Effects Branch/EFED (H7507C)

Attached, please find the EEB review of...

Reg./File # : 053201

Chemical Name : Methyl Bromide  
Type Product : Fumigant  
Product Name : Meth-O-Gas  
Company Name : Chemical Manufacturers Association  
Purpose : Review of fish acute toxicity test (72-1c)

Action Code : 625 6(a) (2) Date Due : 12/25/95  
Reviewer : Tom A. Bailey Date In EEB: 1/25/94

EEB Guideline/MRID Summary Table: The review in this package contains an evaluation of the following:

GDLN NO	MRID NO	CAT	GDLN NO	MRID NO	CAT	GDLN NO	MRID NO	CAT
71-1 (A)			72-2 (A)			72-7 (A)		
71-1 (B)			72-2 (B)			72-7 (B)		
71-2 (A)			72-3 (A)			122-1 (A)		
71-2 (B)			72-3 (B)			122-1 (B)		
71-3			72-3 (C)			122-2		
71-4 (A)			72-3 (D)			123-1 (A)		
71-4 (B)			72-3 (E)			123-1 (B)		
71-5 (A)			72-3 (F)			123-2		
71-5 (B)			72-4 (A)			124-1		
72-1 (A)			72-4 (B)			124-2		
72-1 (B)			72-5			141-1		
72-1 (C)	43066701	P	72-6			141-2		
72-1 (D)						141-5		

Y=Acceptable (Study satisfied Guideline)/Concur  
P=Partial (Study partially fulfilled Guideline but additional information is needed)  
S=Supplemental (Study provided useful information but Guideline was not satisfied)  
N=Unacceptable (Study was rejected)/Nonconcur



## UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

430667-01

## DATA EVALUATION RECORD

D 198212

1. CHEMICAL: Methyl bromide (WIL 2624)
2. TEST MATERIAL: 99.87% TGAI, Pressurized liquid, Lot Number 6RL4. OFFICE OF  
PREVENTION, PESTICIDES  
AND TOXIC SUBSTANCES
3. STUDY TYPE: §72-3
4. CITATION:

Author: Kurt R. Drottar & James P. Swigert, Ph.D.  
 Title: Methyl Bromide: A 96-Hour Static Acute Toxicity Test With The Rainbow Trout (Oncorhynchus mykiss).  
 Date: 17 September 1993 to 16 December 16 1993  
 Laboratory Report #: 264A-105A  
 Any Other Study #: N/A  
 Sponsor: N/A  
 Sponsor #: Methyl Bromide Industry Panel, Chemical Manufacturers Association  
 Laboratory: Wildlife International Ltd., Easton, MD  
 MRID No.: 430667-01

5. REVIEWED BY:

Tom A. Bailey, Senior Aquatic Biologist Signature: *Tom A. Bailey*  
 Ecological Effects Branch  
 Environmental Fate and Effects Division (H7507 C) Date: 9/27/93

6. APPROVED BY:

Henry Craven, Chief, Section 4 Signature: *Henry T. Craven*  
 Ecological Effects Branch  
 Environmental Fate and Effects Division (H7507C) Date: 10/11/94

7. CONCLUSION This study was scientifically sound, but does not fully satisfy the 72-1(c) data requirement. The study design deviated substantially from standard recommended protocols. The study was categorized as "Supplemental". However due to the volatile nature of this product, the data generated is adequate for use in a risk assessment and will not need to be repeated. The LC50 for rainbow trout acutely exposed to methyl bromide is 3.9 mg a.i./L (C.I. 2.9 to 4.6 mg a.i./L). No mortality was observed at concentrations below 2.9 mg a.i./L. This material is considered to be "moderately toxic" to rainbow trout. HTC  
10/28/94

8. RECOMMENDATIONS9. BACKGROUND

2010704



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10. MATERIALS AND METHODSA. Test Organisms:

Guideline Criteria	Reported Information
Species (Scientific Name)	<i>Oncorhynchus mykiss</i>
Mean Weight (0.5-5 grams)	0.13 g (0.1-0.16 g)
Mean Length(S.L. longest not > 2x shortest	23 mm;
Supplier	Mt. Lassen Trout Farm, Red Bluff, CA
All fish from same source (yes or no)	yes
All fish from the same year class (yes or no)	yes
Other Comments	

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period (minimum 14 days)	Not indicated.
Wild caught 7 day quarantine (yes or no)	N/A
Check for signs of disease or injury (yes or no, if yes describe)	No disease reported.
If diseased it can be treated in 48-hr pretest no sign of the disease remains (Report hours prior to test in which no sign of disease or N/A)	N/A
No feeding during the study (When last fed)	Fish not fed.
<3% mortality 48 hours prior to testing (% mortality, if any)	No mortality

C. Test System:



Guideline Criteria	Reported Information
Describe source of dilution water (prefer soft reconstituted water)	Freshwater from 45 m deep well on test site. Medium-hard water.
Does water support test animals without observable signs of stress?	Yes
Was dechlorinated water used (not recommended)	No
Water Temperature (Warm water-17°C or 22°C) (Cold water-12°C)	12°C
pH	7.1 - 8.2
Dissolved Oxygen (Static 1 <sup>st</sup> 48 hrs 60%; 2 <sup>nd</sup> 48 hrs 40%; Flow-through 60%) (% of lowest conc. & hour)	8.0 to > 15 mg/L
Total hardness (40 to 48 mg/L as CaCO <sub>3</sub> well water)	136 mg/L as CaCO <sub>3</sub>
Total Alkalinity	183 mg/L as CaCO <sub>3</sub>
Specific Conductance	333 $\mu$ mhos/cm
Total Organic Carbon	2.2 mg/L
Test Aquaria 1. Material (glass or stainless steel) 2. a. Static volume (18.9 L (5 gal or 19000 cc) with 15 L solution) b. Static or flow-through volume (300x600x300 = 54000 cc.)	1. Clear 4L Serum Bottles 2. 4 L test solution. 3. See 3 page <sup>pgs</sup> (10-12) attachment
Type of Dilution System (Reproducible supply of toxicant)	N/A
Flow rate Consistent flow rate-meter systems calibrated before study and checked 2*24 hours - 5 to 10 vol/24 hours	N/A

Biomass Loading Rate (Static no > 0.8 g/L ≤ 17°C; >17°C 0.5g/L; Flow-through 1 g/L/24)	0.16 g fish per Liter
Photoperiod (16 L & 8 D)	16h L : 8h D
Solvents 1. (Do not exceed 0.5 ml/L for static tests) 2. (Do not exceed 0.1 ml/L for flow-through)	N/A
Other Comments	

D. Test Design:

Guideline Criteria	Reported Information
<u>Range Finding Test</u> (LC <sub>50</sub> >100 mg/L with 30 fish, no definitive test required.)	Not reported.
<u>Definitive Test</u>	
Nominal Concentrations (control+5 treatment levels; dosage should be 60% of the next highest concentration; concentrations should be geometric series)	1.0, 1.7, 2.9, 4.8, 8.0 mg/L
Controls (Minimum control mortality; static 10%; flow-through 5%)	Negative Control
Number of Test Organisms; (Minimum 10/level can be divided among containers)	4 replicates with 5 fish each making total of 20 organisms per concentration.
All organisms must be randomly assigned to test vessels. (yes or no, describe if no)	Trout were removed impartially from acclimation tanks and transferred to test chambers via a funnel.
Biological Observation; (yes or no)	Yes

<b>Water Parameter Measurements</b> 1. Temperature - record every 6 hrs; $>1^{\circ}\text{C}$ . 2. D.O. beginning, 48 hrs, end for control high, medium, and low dose. 3. pH beginning, 48 hrs, end for control, high, medium, and low dose.	1. Measured continuously in a surrogate vessel. 2. Measured in surrogate test chamber at day 0, in controls on day 3, and in all replicates at termination. 3. pH measurements were the same as for D.O.
<b>Chemical Analysis</b> (needed if aeration, volatile, insoluble, precipitate, not steel or glass, known to adsorb, and flow-through) (yes or no)	Yes
<b>Other Comments</b>	Due to high volatility and risk of human exposure, test were run in sealed test vessels.

11. REPORTED RESULTS:

Guideline Criteria	Reported Information
Mean Measured Concentrations (report conc.)	See Attachment No. 1
Recovery of Chemical (% recovery)	See Attachment No. 1
Mortality & Observations (Describe observations & attach mortality tables)	See Attachment No. 2
Author's Comments	

12. STUDY AUTHOR'S CONCLUSIONS / QUALITY ASSURANCE MEASURES:

The 96h LC50 for rainbow trout exposed to five concentrations of methyl bromide was 3.9 mg a.i./Liter. The 95% confidence limits were reported to be between 2.9 and 4.6 mg a.i./Liter. After 96 hours, no mortality was observed at concentrations less than 2.9 mg a.i./Liter.

13. REVIEWER'S DISCUSSION AND INTERPRETATIONA. Test Procedure:

The following items did not meet the guideline



## criteria:

1. The test vessels deviated substantially from that recommended in standard protocols.
2. Tabled data for length and weight measurements were not provided, therefore it was not possible to determine whether the mean standard length of the longest fish was more than 2X the length of the shortest fish.
3. Measurement times for pH, D.O., and temperature were not monitored according to acceptable protocols at periods less than 96 hours. However it is understandable that more frequent measurements were difficult due to the modified test design.
4. The water hardness was significantly greater than the recommended 40 mg/L.
5. There was no mortality between the 16% and 37% levels, which makes determination of an accurate slope difficult.

B. Statistical Analysis

Guideline Criteria	Reported Information
Binomial (yes, no, or not reported)	Yes
Moving Average Angle (yes, no, or not reported)	NO
Probit (yes, no, or not reported)	NO
Other Comments	

C. Discussion/Results: This study was scientifically sound but varied substantially from standard protocol. Because of the nature of these deviations, the study cannot be considered "Core", but will not need to be repeated.

D. Adequacy of the Study:

1. Classification: Supplemental
2. Rationale: Study design deviated substantially from standard guidelines.

430667-01

3. Reparability: Study does not need to be repeated.

14. COMPLETION DATE OF ONE-LINER FOR STUDY:

**Attachments:**

Tom A. Bailey Methyl Bromide Acute Fish Toxicity

\*\*\*\*\*

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
7.7	20	20	100	9.536742E-05
4.6	20	17	85	.1288414
2.9	20	0	0	9.536742E-05
1.9	20	0	0	9.536742E-05
1.3	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 2.9 AND 4.6 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 3.911728

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

\*\*\*\*\*

- 17 -

Table 1  
Summary of Analytical Chemistry Data

Sponsor:		Methyl Bromide Industry Panel Chemical Manufacturers Association		
Test Substance:		Methyl Bromide		
Test Organism:		Rainbow Trout, <i>Oncorhynchus mykiss</i>		
Dilution Water:		Well Water		
Nominal Concentration (mg a.i./L)	Replicate	Measured Concentration (mg a.i./L)		Mean Measured Concentration (mg a.i./L)
		Day 0	Day 4	
Negative Control	A	ND <sup>1</sup>	ND	--
	B	ND	ND	
	C	ND	ND	
	D	ND	ND	
1.0	A	1.61 <sup>2</sup>	1.04	1.3
	B	1.33	1.20	
	C	1.26	1.25	
	D	1.23	1.33	
1.7	A	1.91	2.03	1.9
	B	1.86	2.06	
	C	1.83	2.04	
	D	1.83	1.60	
2.9	A	3.18	2.55	2.9
	B	3.02	2.70	
	C	2.77	2.53	
	D	3.60	2.50	
4.8	A	5.66	4.32	4.6
	B	4.90	3.84	
	C	4.23	4.03	
	D	5.98	4.06	
8.0	A	8.84	6.82	7.7
	B	8.78	6.86	
	C	8.08	7.04	
	D	7.95	6.99	

<sup>1</sup> Not detected; peak area response was less than the lowest calibration standard (0.51 mg methyl bromide/L).

<sup>2</sup> Values are the calculated means of the two injections for each replicate.

Table 3  
Cumulative Mortality and Treatment-Related Effects

Sponsor:		Methyl Bromide Industry Panel Chemical Manufacturers Association											
Test Substance:		Methyl Bromide											
Test Organism:		Rainbow Trout, <i>Oncorhynchus mykiss</i>											
Dilution Water:		Well Water											
Mean Measured Concentration (mg a.i./L)	Replicate	No. Exposed	14 Hours		24 Hours		48 Hours		72 Hours		96 Hours		Cumulative Percent Mortality
			No. Dead <sup>1</sup>	Effects <sup>2</sup>	No. Dead	Effects	No. Dead	Effects	No. Dead	Effects	No. Dead	Effects	
Negative Control	A	5	0	SAN	0	SAN	0	SAN	0	SAN	0	SAN	0%
	B	5	0	SAN	0	SAN	0	SAN	0	SAN	0	SAN	
	C	5	0	SAN	0	SAN	0	SAN	0	SAN	0	SAN	
	D	5	0	SAN	0	SAN	0	SAN	0	SAN	0	SAN	
1.3	A	5	0	SAN	0	SAN	0	SAN	0	SAN	0	SAN	0%
	B	5	0	SAN	0	SAN	0	SAN	0	SAN	0	SAN	
	C	5	0	SAN	0	SAN	0	SAN	0	SAN	0	SAN	
	D	5	0	SAN	0	SAN	0	SAN	0	SAN	0	SAN	
1.9	A	5	0	SAN	0	SAN	0	SAN	0	SAN	0	SAN	0%
	B	5	0	SAN	0	SAN	0	SAN	0	SAN	0	SAN	
	C	5	0	SAN	0	SAN	0	SAN	0	SAN	0	SAN	
	D	5	0	SAN	0	SAN	0	SAN	0	SAN	0	SAN	
2.9	A	5	0	SAN	0	SAN	0	SAN	0	3C	0	1D, N, 4C	0%
	B	5	0	SAN	0	SAN	0	1C, 1D, 3AN	0	1AN, 1C, N, 3C	0	1D, 1C, N, 3C, D	
	C	5	0	SAN	0	SAN	0	2C, 3AN	0	5C	0	5C	
	D	5	0	SAN	0	SAN	0	SAN	0	2AN, 3C	0	1D, N, 4C, D	
4.6	A	5	0	SAN	0	SAN	1	2N*, 2C, D, N	2	3N*	5	N/A	85%
	B	5	0	SAN	0	SAN	0	5C, N	1	4N*	5	N/A	
	C	5	0	SAN	0	SAN	1	1N*, 3C, N	2	2N*, 1C, N	2	3N*	
	D	5	0	SAN	0	SAN	0	2N*, 3C, D, N	2	2N*, 1C, N	5	N/A	
7.7	A	5	0	SAN	1	4C, N	5	N/A	5	N/A	5	N/A	100%
	B	5	0	SAN	0	5C, N	5	N/A	5	N/A	5	N/A	
	C	5	0	SAN	1	4C, N	5	N/A	5	N/A	5	N/A	
	D	5	0	SAN	0	5C, N	5	N/A	5	N/A	5	N/A	

<sup>1</sup> Cumulative number of dead fish.

<sup>2</sup> Observed Effects: AN = Appears Normal; C = Lethargy; D = Discoloration; N = Loss of equilibrium; N\* = Lying on side with only gill movement.  
N/A = not applicable due to 100% mortality.



## TRANSMITTAL DOCUMENT

TO: Lois Rossi  
Reregistration Branch  
Special Review and Reregistration Division  
c/o Document Processing Desk (RS-0647)  
Crystal Mall II, Room 266A  
1921 Jefferson Davis Highway  
Arlington, VA 22202

1. Name and Address of Submitter

Chemical Manufacturers Association  
2501 M Street, N.W.  
Washington, D.C. 20037

2. Regulatory Action in Support of which this Package is Submitted

The Data Call-In Notice for Methyl Bromide, EPA, OPP, September 20, 1991, Case No. 0335.

3. Transmittal Date

December 23, 1993

4. Studies Submitted (three copies each enclosed)

Methyl Bromide: A 96-Hour Static Acute Toxicity Test With The  
Rainbow Trout 43066701

(In Support of Guideline No. 72-1(c))

CHEMICAL MANUFACTURERS ASSOCIATION  
METHYL BROMIDE INDUSTRY PANEL

Ameribrom, Incorporated  
52 Vanderbilt  
New York, NY 10017

Ethyl Corporation  
451 Florida Street  
Baton Rouge, LA 70801

Great Lakes Chemical Corporation  
P.O. Box 2200  
US Highway 52 NW  
West Lafayette, IN 47906

Trical, Incorporated  
P.O. Box 1327  
Hollister, CA 95024

Chemical Manufacturers Association  
December 23, 1993 Transmittal Document  
Page 2

Panel Official: Kathryn A. Rosica

Kathy Rosica  
Signature

Panel Name:

Chemical Manufacturers Association  
Methyl Bromide Industry Panel

(A list of Methyl Bromide Industry Panel members  
is attached.)

Panel Contact:

Kathryn A. Rosica (202)887-1293